The Breast (2005) **14**, 71–74

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CASE REPORT

ETV6–NTRK3 gene fusion in a secretory carcinoma of the breast of a male-to-female transsexual

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Received 20 January 2004; received in revised form 25 March 2004; accepted 15 April 2004

KEYWORDS
Secretory Carcinoma;
ETV6–NTRK[3] gene fusion;
Male breast cancer

Summary Secretory carcinomas of the breast were first described as ''juvenile carcinoma'' by McDivitt and Stewart in a cohort of children. This term has been replaced by the term ''secretory breast carcinoma'', because the entity can occur at any time of life. Carcinoma of the male breast is uncommon and accounts for approximately 1% of all cancers in men. Recently, it has been reported that human secretory breast carcinoma expresses the *ETV6–NTRK3* gene fusion that was previously cloned in pediatric mesenchymal cancers. We present the case of a 46-year-old male-to-female transsexual in whom a secretory breast carcinoma was an incidental finding. As confirmation of the histopathological diagnosis we detected the novel *ETV6–NTRK3* gene fusion in this tumor.

Introduction

Secretory breast carcinoma is a rare, indolent form of breast cancer. Approximately 100 cases of this entity have so far been published in a small number of studies. This neoplasm was originally described in 1966 by McDivitt and Stewart[1] as an uncommon variety of mammary carcinoma occurring in children, which they designated as ''juvenile carcinoma''. Subsequent studies have demonstrated that the lesion also occurs in older individuals, including a substantial number of postmenopausal women. Altogether, approximately two-thirds of all published cases have been in adults,[2,3] though the average age of the patients is significant lower than in more common forms of breast carcinoma.

Patients with secretory carcinoma have an exceptionally good prognosis. It is a slow-growing type of breast carcinoma with low malignant potential.[4]

Human secretory breast carcinoma was recently found to express the *ETV6–NTRK3* gene fusion[5] previously cloned in pediatric mesenchymal cancers.[6] This gene fusion encodes a chimeric tyrosine kinase with potent transforming activity. This represents a recurrent chromosomal rearrangement and expression of a dominantly acting

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0960-9776/$ - see front matter © 2004 Published by Elsevier Ltd.
doi:10.1016/j.breast.2004.04.005

oncogene as a primary event in human secretory breast carcinoma. *ETV6–NTRK3* expression has been confirmed in up to 92% of secretory breast carcinomas but not detected in other ductal carcinomas.

Case report

Clinical course

A 46-year-old male-to-female transsexual patient presented with the clinical signs of silicone breast implant dislocation and pain in the left breast. In 1986, she had undergone sexual reassignment surgery (bilateral orchiectomy, penectomy, vaginoplasty, and augmentation mammaplasty) in Thailand. She also explained that she had undergone long-term cross-sex hormone treatment, without, however giving any details about choice, dosage, or duration. Her grandmother had died of cancer of an unknown type, and the patient has been a smoker for years.

Gross pathology

An intact tissue specimen ($4.0 \times 2.0 \times 2.0$ cm and 14 g) was obtained from the patient's left breast during surgical reexploration performed because of silicone gel breast implant dislocation. Initially the specimen was regarded as a removed siliconoma. The cross-section showed a $3.5 \times 2.0 \times 1.8$-cm tumor that was grayish-white to tan in color and had a center that varied between a spongy consistency and a microcystic nature. The margin of the lesion was ill defined. During section a colorless viscous secretion emerged from the central tissue compartment (Fig. 1A).

Histopathology

Investigation of the paraffin-embedded material revealed an incompletely resected tumor made up predominantly of microcystic and to a lesser extent of solid or follicular areas. The tumor had a lobulated appearance because of a connective tissue network. The most characteristic feature of the tumor was an intra- and extracellular secretion. The cystic spaces contained abundant secretion product, which usually stained pale pink with hematoxylin and eosin and were strongly positive with the periodic acid-Schiff reaction (Fig. 1B). Minor nuclear pleomorphism was observed in the neoplasm, and only a few mitoses could be detected.

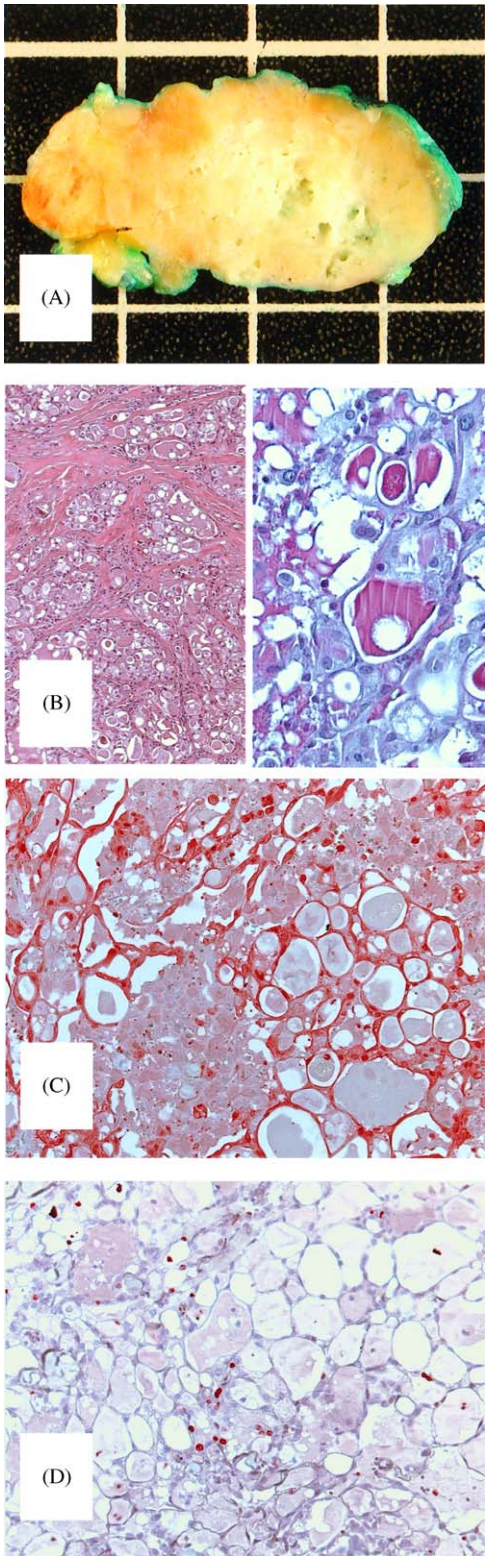

Figure 1 (A) Cross-section of the tumor. (B) (Left) Hematoxylin and eosin-staining (low power), (Right) periodic acid-Schiff stain (high power). Immunohistochemical stains of the tumor tissue. (C) S-100. (D) MIB-1.

Immunohistochemistry

The most important immunohistochemical features of the tumor were a strong immunopositivity for pancytokeratin and S100 (Fig. 1C). The labeling index of MIB-1 was less than 5% (Fig. 1D). Estrogen, androgen, and progesterone receptors were not expressed on tumor cells, whereas adjacent non-neoplastic ductal epithelium showed variably strong nuclear staining.

All the immunohistochemical findings are shown in Table 1 with the characteristics of the antibodies used.

RT-PCR analysis of the tumor and sequencing

RT-PCR analysis of the secretory breast carcinoma confirmed *ETV6–NTRK3* expression, whereas in three other tumor entities of the breast this gene fusion was not detectable (fibroadenoma, invasive ductal and invasive lobular type of carcinoma, Fig. 2, top). The sequence analysis of the purified RT-PCR-product revealed the *ETV6–NTRK3* gene fusion (Fig. 2, bottom).

Materials and methods

RT-PCR analysis of tumor samples

Total ribonucleic acid (RNA; 2 μg) was isolated from frozen tissue samples taken of the tumors using the RNeasy® kit (Qiagen, Germany) and was converted to complementary deoxyribonucleic acid (cDNA) by M-MuLV reverse transcriptase (MBI Fermentas, Germany). The cDNA samples were then subjected to PCR using the sense primer TEL971 and the antisense primer TRKC1059.[7] PCR conditions were as follows: 94°C for 10 min, followed by 33 cycles of 94°C for 45 s, 60°C for 1 min, 72°C for 1 min, and a

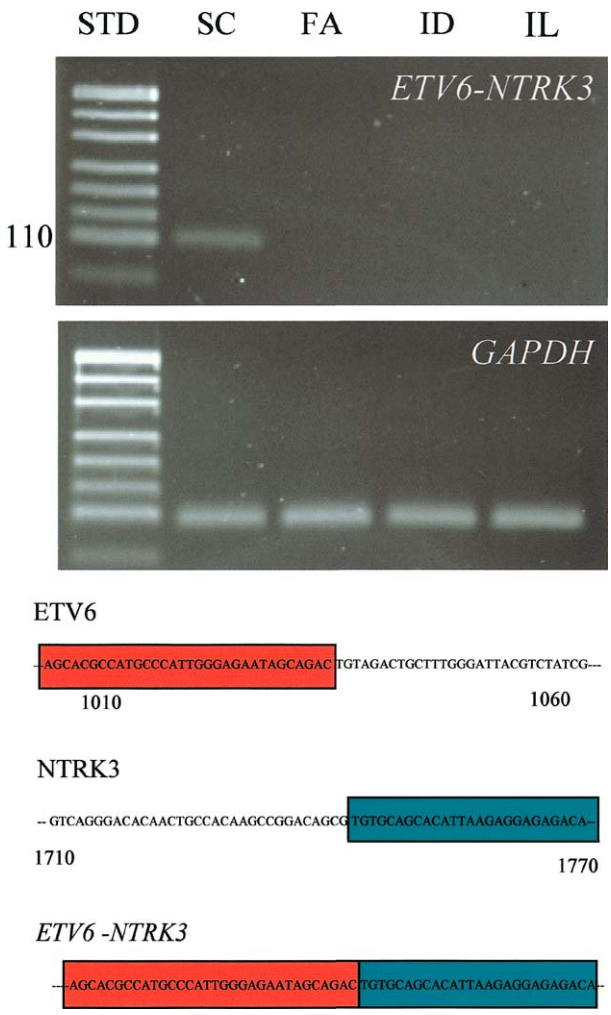

Figure 2 Top: Gel electrophoresis of the RT-PCR products of four breast tumors. Only the SC shows the 110-bp RT-PCR product of the *ETV6–NTRK3* fusion (sense primer TEL971 and antisense primer TRKC1059). The presence of amplifiable RNA was confirmed by RT-PCR using control primers (GAPDH). Bottom: Sequencing of the SC-RT-PCR product revealed the typical *ETV6–NTRK3* fusion. SC = Secretory carcinoma; FA, fibroadenoma; ID, invasive ductal carcinoma; IL, invasive lobular carcinoma).

Table 1 Immunohistochemical findings.

Antibody	Reactivity	Dilution	Clone	Source
Pan-cytokeratin	+	Ready	AE1, Ae3, Ks13.1	Linaris
S100	+	1:500	Polyclonal	Dako
Leu-M1 (CD15)	+	1:50	C3D-1	Dako
Androgen receptor	–	1:50	AR 441	Dako
Carcinoembryonic antigen	–	1:100	Polyclonal	Dako
Estrogen receptor-α	–	1:25	1D5	Dako
Progesterone receptor	–	1:50	PgR636	Dako
HER2	–		HercepTest™	Dako
MIB-1	<5%	Ready	MIB-1	BioGenex

final extension of 72°C for 10 min. The presence of amplifiable RNA in all samples was confirmed by RT-PCR using control primer sets (GAPDH).

Sequencing of RT-PCR-product

The RT-PCR-product was purified by using the QIAquick® PCR purification kit (Qiagen, Germany) and was sequenced with the ABI Prism® Big Dye™ Terminator Cycle Sequencing Kit (PE Biosystems, USA) according to the manufacturers' recommendations.

Discussion

Secretory carcinomas of the breast are very rare tumors. This entity was originally described as "juvenile carcinoma" by McDivitt and Stewart in a cohort of seven children.[1] Meanwhile this term has been replaced by "secretory breast carcinoma", because about two thirds of all published cases have been in adults.[2]

Carcinoma of the male breast is uncommon and accounts for under 1% of all cancers in boys and men and 1% of all breast cancers.[8,9] Almost all of the histological subtypes of breast cancer that have been described in girls and women have also been reported in male subjects. Lobular carcinoma is much less common in males, and for a long time it was thought not to affect men because of the absence of terminal duct lobular units in men.[9] Secretory carcinomas of the male breast have occasionally been reported in adults and children.[4]

A search of the literature from 1966 to 2003 revealed three publications with altogether four cases of breast carcinoma (invasive ductal type) in male-to-female transsexuals 30–36 years of age. All these patients received a long-term (5–14 years) hormone replacement therapy. Numerous studies have investigated the effects of sex steroid hormones used for contraception and hormone replacement therapy, but epidemiological evidence of the relationship between sex hormones and breast cancer remains controversial.[10,11]

While the risk factors for breast cancer in males are known to include abnormalities in estrogen and androgen balance,[9] the relationship between sex hormones and the development of (secretory) carcinomas is not clear.[2,4] Studies dealing with the mortality and morbidity in transsexuals treated with cross-sex hormones show that the overall mortality is no higher than in the general population and that by and large the observed mortality cannot be linked to hormone treatment.[12]

Recently the expression of *ETV6–NTRK3* gene fusion was described in human secretory carcinomas of the breast.[5] This gene fusion was previously cloned in the pediatric solid tumor congenital fibrosarcoma.[6] *ETV6–NTRK3* expression has been detected exclusively in cases of secretory carcinomas, and not in other ductal breast carcinomas investigated.[5]

The rare diagnosis of secretory carcinoma was confirmed in this specific case by the identification of this novel gene fusion. Three other benign and malignant breast tumors were negative for it.

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